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Analysis of *KIT*, *SCF*, and Initial Screening of *SLUG* in Patients with Piebaldism

To the Editor:

Piebaldism is an autosomal dominantly inherited disorder characterized by congenital leukoderma, typically on the forehead, abdomen, and knees. The leukoderma is usually stable throughout life, although pigmented macules may develop at the margins and even within the white macules (Fukai *et al*, 1989). *KIT* mutations have been demonstrated in about 75% of patients with piebaldism (Ezoe *et al*, 1995). A mouse model for human piebaldism, *W* dominant white spotting, similarly results from mutations involving the murine *Kit* locus. Mutations of stem cell factor in steel (*sl*) mutant mice are associated with *W*-like abnormalities of pigmentation (Chabot *et al*, 1988; Geissler *et al*, 1988). In addition, deletions in the *SLUG* gene, which is a zinc-finger neural crest transcription factor, have been reported recently in human piebaldism that lacked mutations in *KIT* (Sanchez-Martin *et al*, 2003).

From 1998 to 2003, we had the opportunity to examine 30 families with human piebaldism. Eight novel mutations found were already reported elsewhere (Richards *et al*, 2001; Murakami *et al*, 2004; Shears *et al*, 2004). Here, we report the result of the analysis of the remaining 22 families with piebaldism.

Genomic DNA was prepared from peripheral blood leukocytes. All 21 exons and flanking intron sequences of the *KIT* gene were amplified by PCR as described (Giebel *et al*, 1992). PCR products were gel-purified and direct-sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California) and an ABI310 automated sequencer (PE Applied Biosystems, Tokyo, Japan). This work was approved by the ethics committee of Osaka City University Graduate School of Medicine (#135), and was conducted according to the Declaration of Helsinki Principles. As shown in Table I, we found six mutations in *KIT* gene, which were predicted to be pathological. Among these, five mutations were novel: 358-delG, IVS3-2A>G, Q346X, H650L, and D792Y (Table I).

Family 1 showed 358delG frameshift mutation, which would result in translational truncation at codon 143, within the ligand-binding domain. Therefore, only the KIT proteins expressed from the normal allele can work. The relatively mild piebaldism phenotype associated with this mutation results from haploinsufficiency (Spritz, 1994).

Family 2 had a heterozygous transition at codon 136, which substitutes Arg for Cys (C136R). The strongly basic charge of the arginine within the extracellular domain appears likely to impair ligand binding. The same mutation has been reported previously (Fleischman *et al*, 1996).

Family 3 had a splice site mutation in intron 3 (IVS3-2A>G). Peptides from this abnormal allele should have normal amino acid sequences until codon 206, approximately two-fifths of the ligand-binding domain. If new splicings occur to result in in-frame deletions or insertions, the new KIT peptides should have impaired ligand binding. Otherwise, the new KIT peptides should be followed by meaningless stretches of amino acids, and terminates within the ligand-binding domain. Therefore, this mutation results in haploinsufficiency.

Family 4 had a nonsense mutation at codon 346 (Q346X) that leads to truncation of the KIT receptor within the ligand-binding domain, and also to nonsense-mediated mRNA decay and haploinsufficiency.

Family 5 had a heterozygous missense substitution at codon 650 (H650L) within the tyrosine kinase domain. Recently, the crystal fine structure of the active KIT tyrosine kinase was determined (Mol *et al*, 2003). It is composed of a smaller amino-terminal N-lobe, which is comprised of mostly β-sheets, and a larger predominantly α-helical carboxy-terminal C-lobe. The KIT active site is located in the interdomain cleft between the N- and C-lobes. The N-lobe has a single α-helical structure, called helix αC, which directly contacts the A-loop DFP motif and nucleotide binding site and modulates kinase activity. The His650, mutated for leucine in this family 5, is located in a strand connecting the helix αC and the β-sheet, three amino acids downstream of the helix αC. This histidine faces one of the α-helical structures of the C-lobe. It is most conceivable that the substitution of basic and hydrophilic His650 for Leu, which is neutral and hydrophobic, distorts the conformation of the active KIT protein. As the mutation at the same codon 650 (H650P) was reported to result in a severe phenotype because of a dominant-negative effect (Ezoe *et al*, 1995), the severe phenotype in this family 5 is also likely to be caused by the dominant-negative effect.

Family 6 had a heterozygous missense substitution at codon 792 (D792Y) within the tyrosine kinase domain. The Asp792 is located in the center of the interdomain cleft of the N- and C-lobes. The substitution of neutral Leu for acidic Asp within the cleft may have a substantial effect on the association of the substrate peptide with the cleft. The severe phenotype of family 6 is probably caused by the dominant-negative effect (Spritz, 1994). In 50 unaffected and unrelated subjects, the above six missense mutations were not observed, and therefore are unlikely to be polymorphisms.

Abbreviation: SCF, stem cell factor

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Table I. Analysis of KIT, SCF, and SLUG genes in human piebaldsim

Family	Country	Age/sex	Phenotype	Result of the KIT	Result of the SCF	Result of the SLUG
1	Japan	1 M	Mild	358delG (exon3)	ND	ND
2	USA	Child F	Mild	C136R (exon3)	ND	ND
3	Italy	38 M	Moderate	IVS3-2A > G	ND	ND
4	Italy	Child M	Mild	Q346X (exon6)	ND	ND
5	France	44 M	Severe	H650L (exon13)	ND	ND
6	Italy	Child F	Severe	D792Y (exon17)	ND	ND
7	the Netherlands	43 F	Moderate	No mutation	No mutation	No mutation
8	Japan	28 F	Moderate	No mutation	No mutation	No mutation
9	Japan	0 M	Severe	No mutation	No mutation	No mutation
10	England	6 F	Mild	No mutation	No mutation	No mutation
11	Japan	Adult F	Severe	No mutation	No mutation	No mutation
12	USA	6 F	Mild	No mutation	No mutation	No mutation
13	Japan	37 M	Mild	No mutation	No mutation	No mutation
14	England	23 F	Mild	No mutation	No mutation	No mutation
15	Canada	7 M	Unknown	No mutation	No mutation	No mutation
16	England	39 F	Moderate	No mutation	No mutation	No mutation
17	USA	28 F	Severe	No mutation	No mutation	No mutation
18	England	16 F	Unknown	No mutation	No mutation	No mutation
19	Italy	4 F	Moderate	No mutation	No mutation	ND
20	Colombia	11 M	Moderate	No mutation	No mutation	ND
21	England	43 F	Mild	No mutation	No mutation	No mutation
22	USA	1 F	Moderate	No mutation	ND	ND

SCF, stem cell factor; M, male; F, female; ND, non-determinant.

For *KIT*-mutation-negative cases, the nine exons and flanking introns of *stem cell factor* (*SCF*) gene (Ezoe *et al*, 1995) were also analyzed in the same manner as *KIT* gene. But, we were not able to find mutations in the *SCF* gene. In mice, a mutation in *SCF* or *steel factor* causes piebald phenotype (Chabot *et al*, 1988; Geissler *et al*, 1988). Despite the extensive analysis by Ezoe and us, however, mutations in the *SCF* gene were not identified in human piebaldism. Mutations in *SCF* probably have no phenotypic effect in humans, or could be lethal in humans.

For *KIT–SCF*-mutation-negative cases, we further screened the *SLUG* gene. Five regions of the *SLUG* gene were first screened by single strand conformation polymorphism, and PCR products that showed aberrant bands were subjected to direct-sequencing. The primer pairs used will be obtained as supplementary data on line. In this screening system, we could not find any mutations in the *SLUG* gene.

Entire or partial deletions of the *KIT* gene (Ezoe *et al*, 1995), deletions of both *KIT* and the *PDGFRA* gene (Fleischman *et al*, 1991; Spritz *et al*, 1992), and deletions of the *SLUG* gene (Sanchez-Martin *et al*, 2003) have been reported in human piebaldism. Southern blotting has been the method of first choice for detecting large deletions. We have three genes to analyze for human piebaldism, however, and the quantity of genomic DNA from most of the patients we have is not enough for three Southern blotting analyses. Because real-time quantitative PCR requires only a small amount of genomic DNA, the establishment of a method to detect large deletion(s) of *KIT, SCF*, and *SLUG* genes is underway.

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This work was supported by a grant-in-aid from Ministry of Education in Japan #11670846 (to K. F.).

Supplementary Material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/JID/JID23637/JID23637sm.htm
Supplementary data. Primer sequences for amplifying exons and flanking introns of slug gene

DOI: 10.1111/j.0022-202X.2005.23637.x

Manuscript received October 17, 2004; revised November 19, 2004; accepted for publication November 23, 2004

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